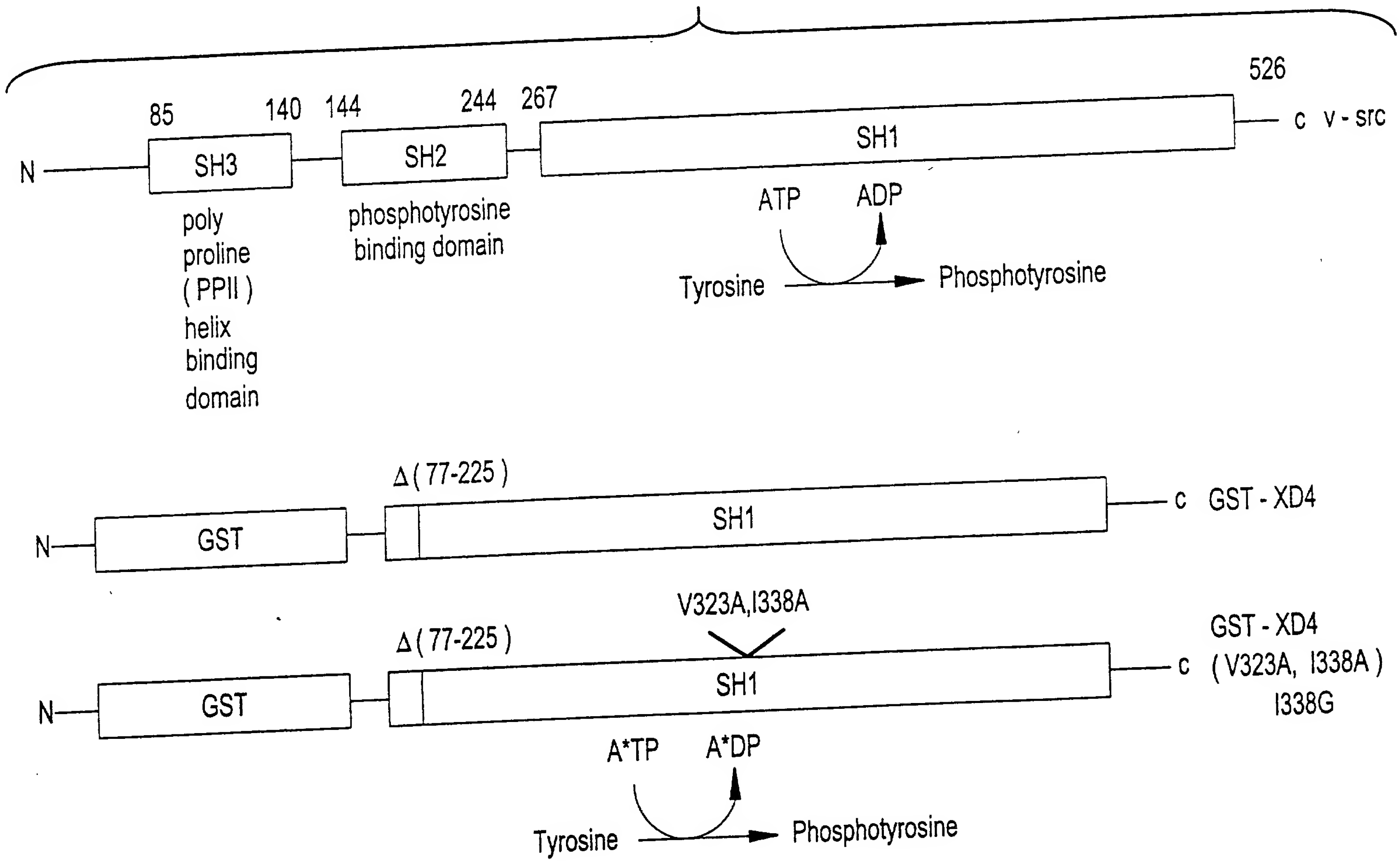
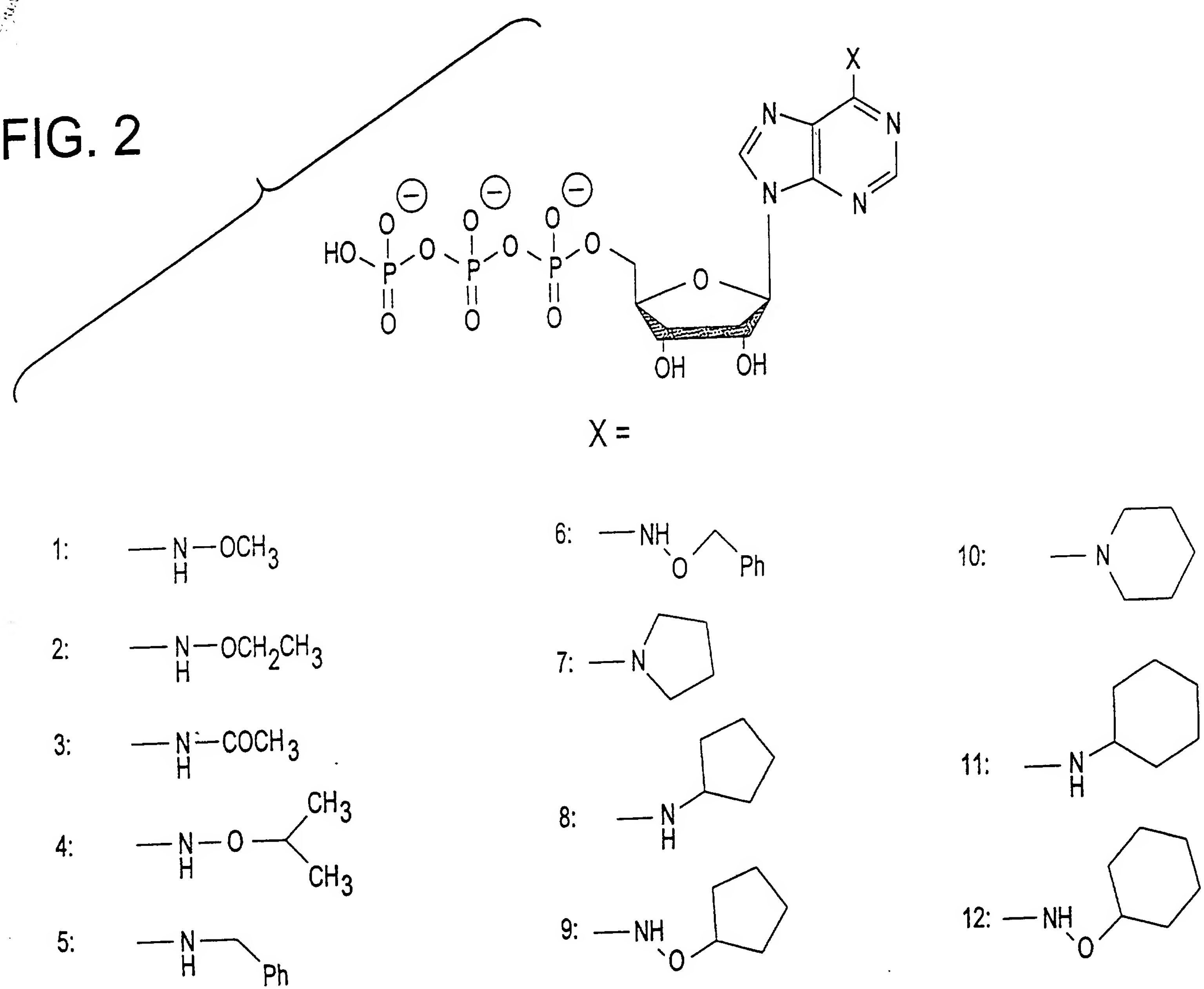
FIG. 1



N
85
SH3
140
144
SH2
244
267
SH1
526
c
v - src
poly
proline
(PPII)
helix
binding
domain
phosphotyrosine
binding domain
ATP
ADP
Tyrosine
Phosphotyrosine
N
GST
Δ (77-225)
SH1
c
GST - XD4
N
GST
Δ (77-225)
V323A,I338A
SH1
c
GST - XD4
(V323A, I338A)
I338G
A*TP
A*DP
Tyrosine
Phosphotyrosine

FIG. 2



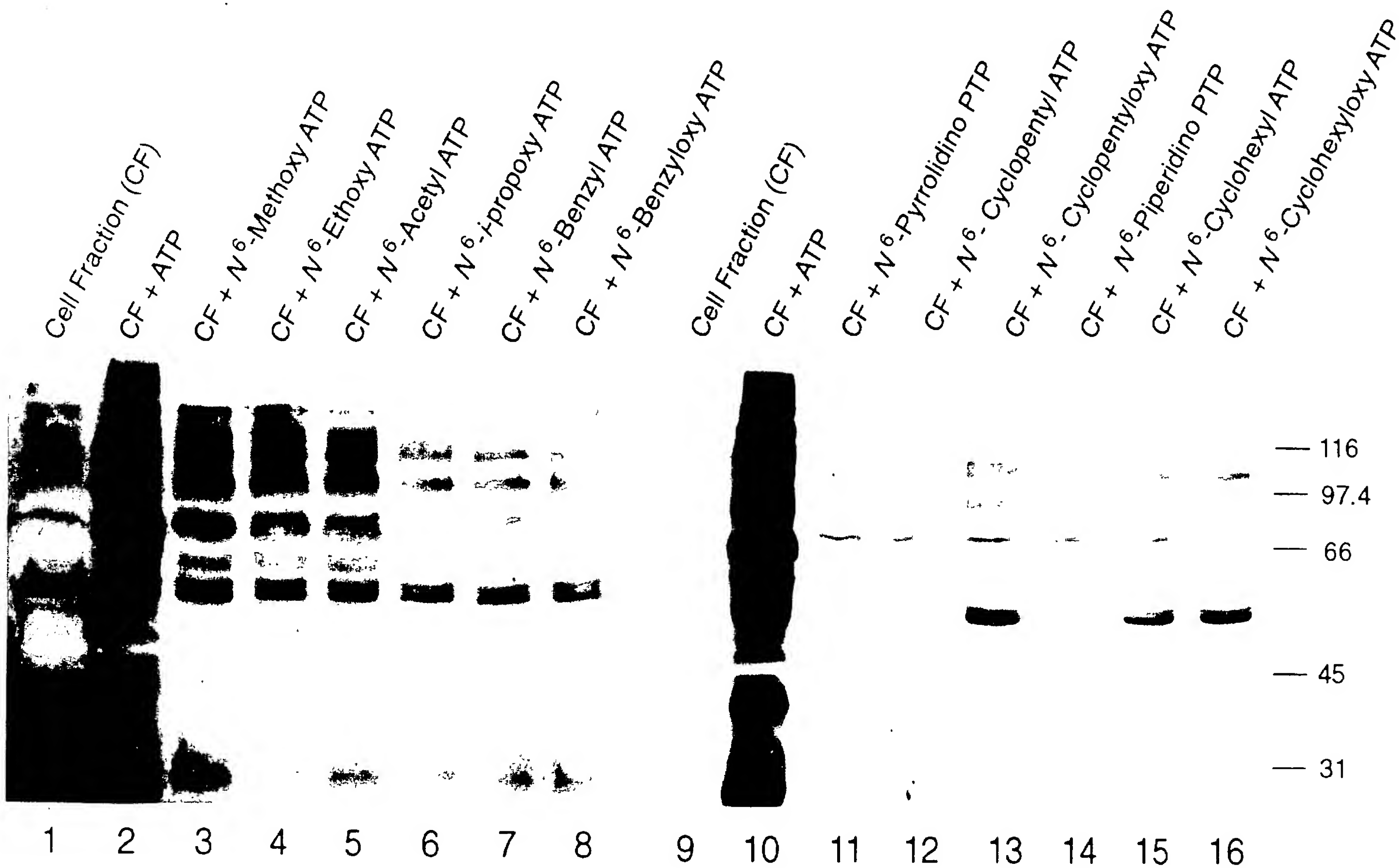

Cell Fraction (CF)
CF + ATP
CF + N 6-Methoxy ATP
CF + N 6-Ethoxy ATP
CF + N 6-Acetyl ATP
CF + N 6-i-propoxy ATP
CF + N 6-Benzyl ATP
CF + N 6-Benzyloxy ATP
Cell Fraction (CF)
CF + ATP
CF + N 6-Pyrrolidino PTP
CF + N 6- Cyclopentyl ATP
CF + N 6- Cyclopentyloxy ATP
CF + N 6-Piperidino PTP
CF + N 6-Cyclohexyl ATP
CF + N 6-Cyclohexyloxy ATP
116
97.4
66
45
31
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16

FIG.3

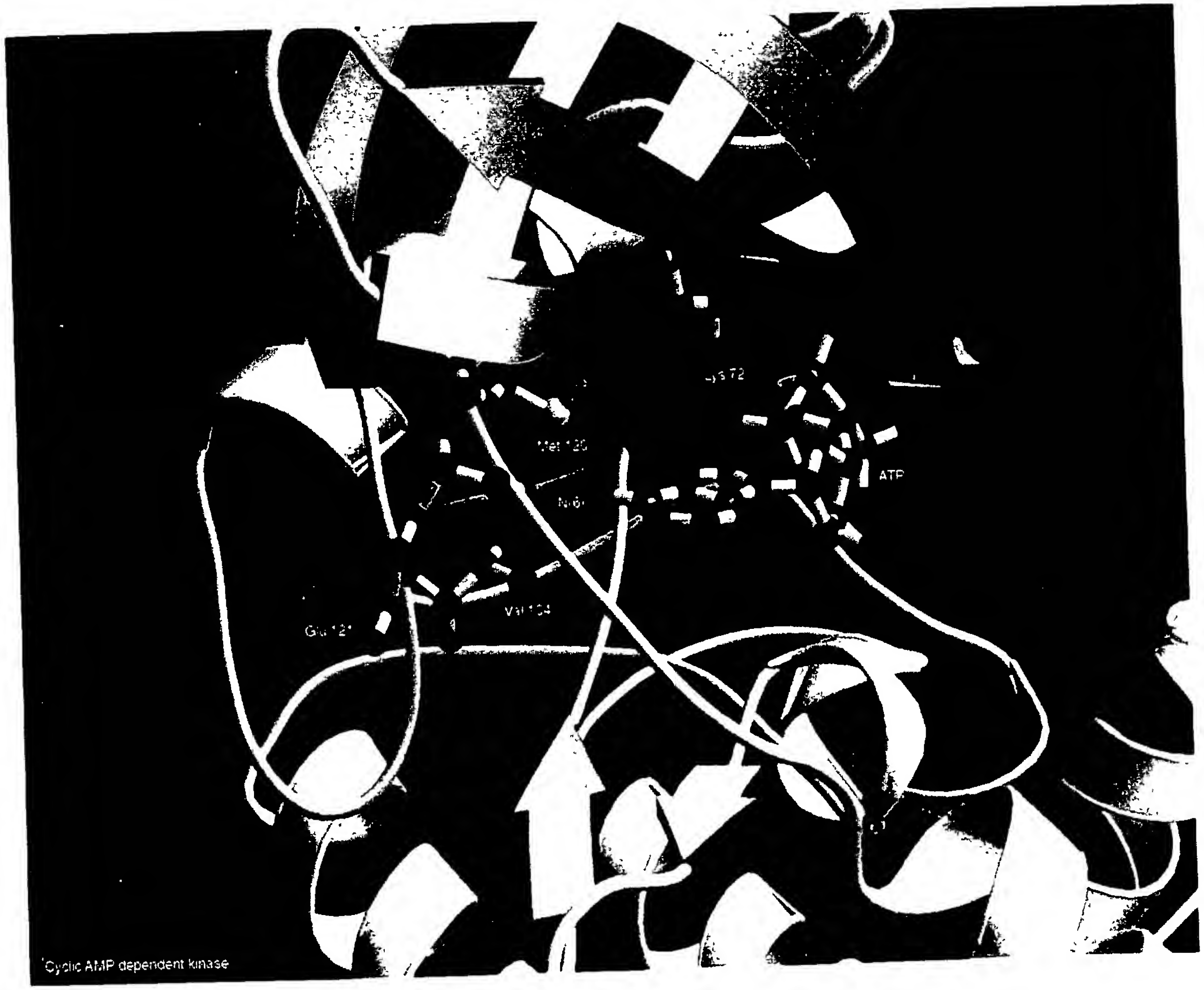


FIG.4

FIG.5A

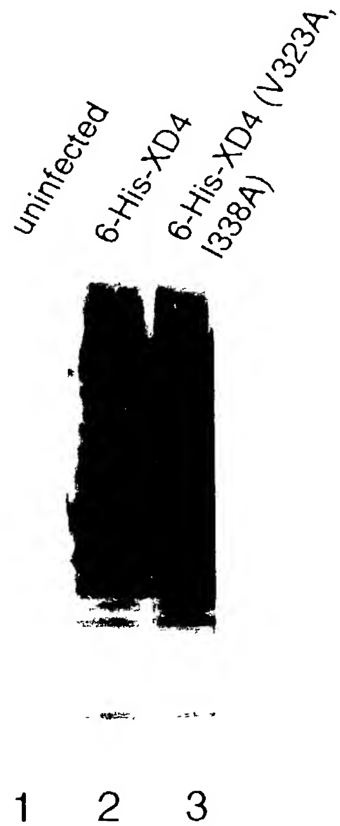


FIG.5B

FIG.5C

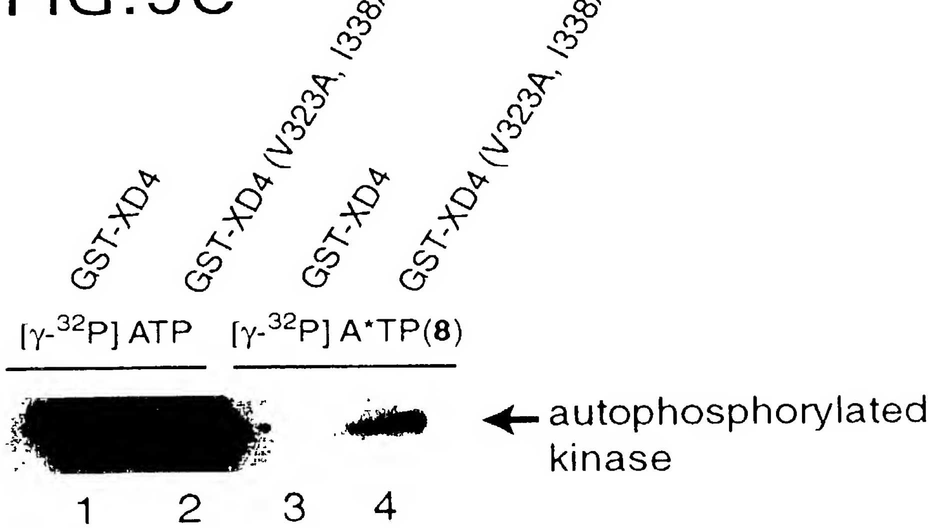


FIG. 6

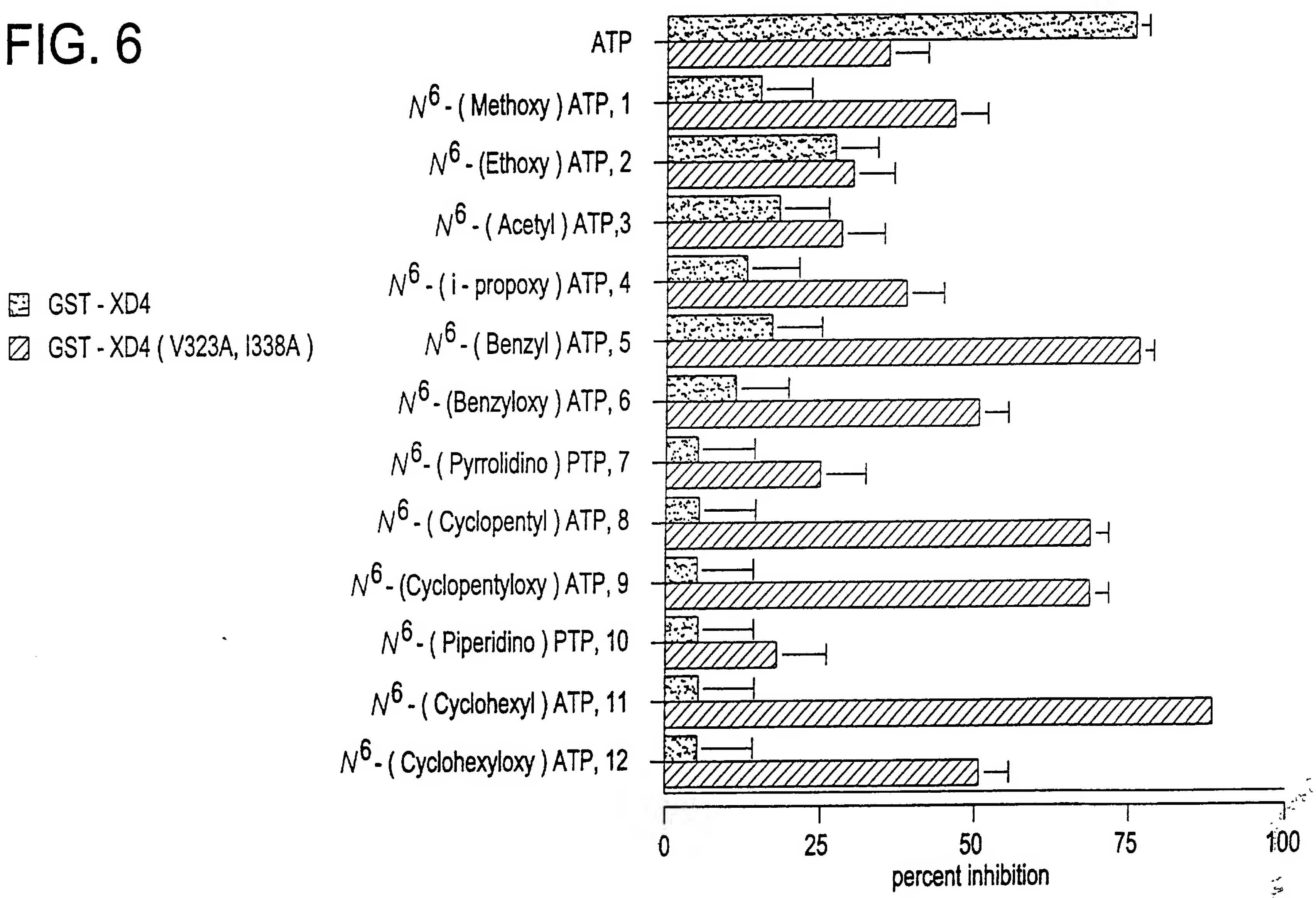

GST - XD4
GST - XD4 (V323A, I338A)
ATP
N6 - (Methoxy) ATP, 1
N6 - (Ethoxy) ATP, 2
N6 - (Acetyl) ATP,3
N6 - (i - propoxy) ATP, 4
N6 - (Benzyl) ATP, 5
N6 - (Benzyloxy) ATP, 6
N6 - (Pyrrolidino) PTP, 7
N6 - (Cyclopentyl) ATP, 8
N6 - (Cyclopentyloxy) ATP, 9
N6 - (Piperidino) PTP, 10
N6 - (Cyclohexyl) ATP, 11
N6 - (Cyclohexyloxy) ATP, 12
0
25
50
75
100
percent inhibition

FIG.7A

[γ-^{32}P] ATP

I338A I338S

FIG.7B

[γ-^{32}P] N^{6}-cyclopentyl ATP

I338A I338S

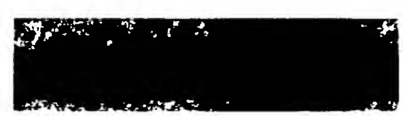
FIG.7C

[γ-^{32}P] ATP

I338A I338G

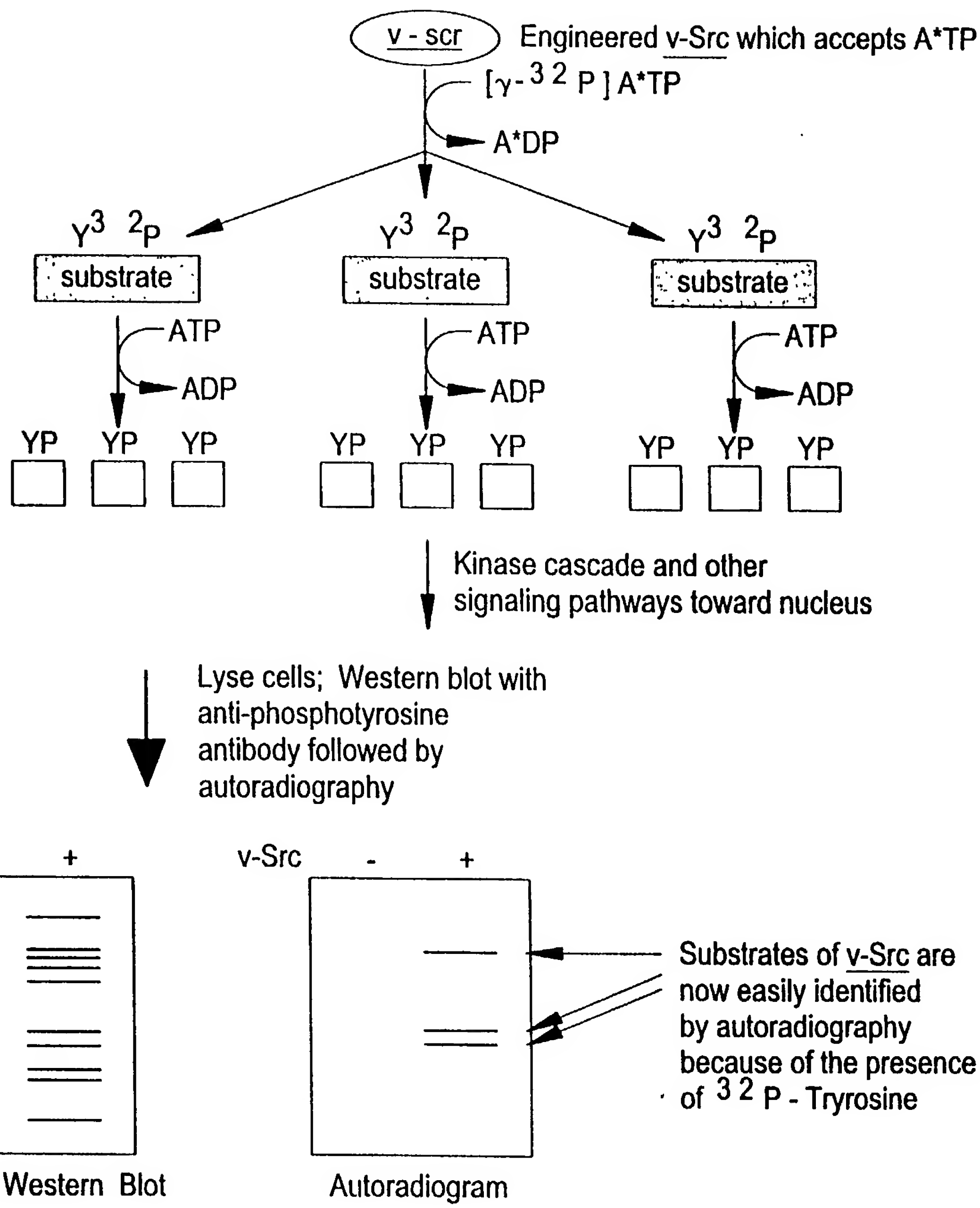
FIG.7D

[γ-^{32}P] N^{6}-cyclopentyl ATP



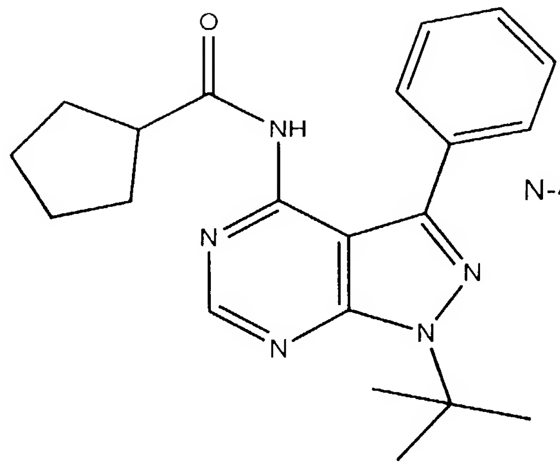
I338A I338G

FIG. 8



v - scr
Engineered v-Src which accepts A*TP
[γ-32 P] A*TP
A*DP
Y32P
substrate
ATP
ADP
YP
Kinase cascade and other signaling pathways toward nucleus
Lyse cells; Western blot with anti-phosphotyrosine antibody followed by autoradiography
v-Src
-
+
Anti - PY Western Blot
Autoradiogram
Substrates of v-Src are now easily identified by autoradiography because of the presence of 32 P - Tyrosine

FIG. 11A



N-4 cyclopentoyl PP3

FIG. 11B

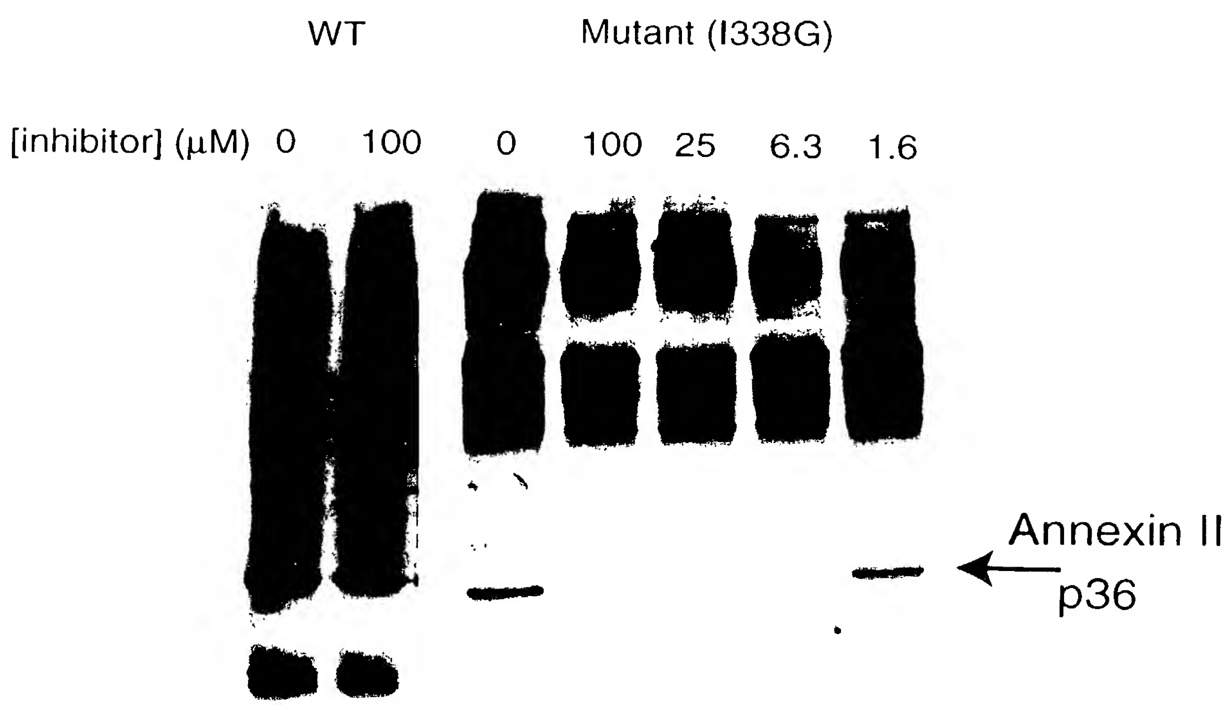


FIG. 12A

IC_{50} (μM)

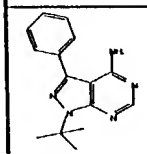
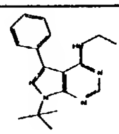
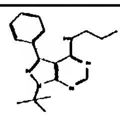
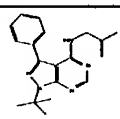
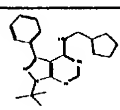
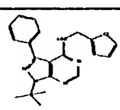
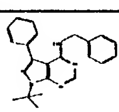
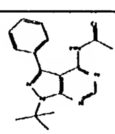
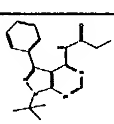
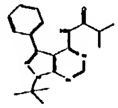
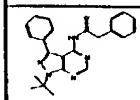
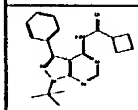
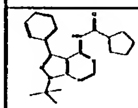
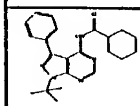
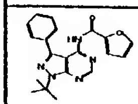
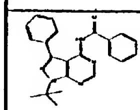
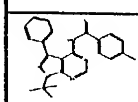
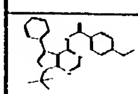
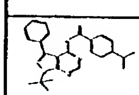
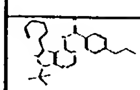
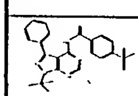
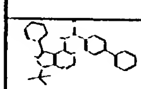
	Molecule	WT XD4	I338G XD4	WT Fyn	T339G Fyn	WT Abl	T120A Abl
a		35	0.13	0.05			<<10
b			200	>300			
c			300	>300			
d			>300	>300			
e		>300	75	>300	100		>10
f		>300	250	>300	26		>10
g		>300	85	>300	63		>10
h							
i							

FIG. 12B

j							
k							
l		>300	12	6.5	5		
m		>300	19	80	9		
n		>300	20	50	5		
o		>300	150	15	19		
p		>300	10	300	11		(10
q		>300	10	300	6		(10
r			1.2				<10
s			0.63				
t			(0.411				1.8
u		>300	0.43	300	0.83	300	(10
v							

10094967 .052902

FIG. 12C

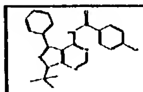
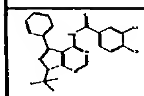
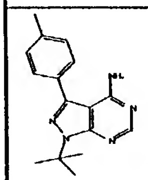
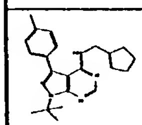
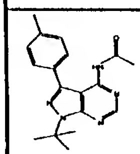
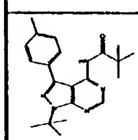
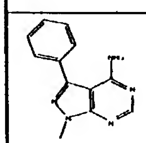
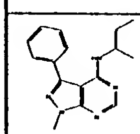
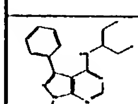
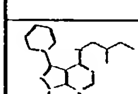
w							
x							>10
y		100	(0.05	0.1			
z			>100	>300			
aa				2			
bb				7			
cc							
dd							
ee							
ff							

FIG. 12D

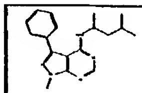
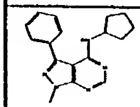
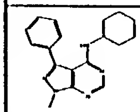
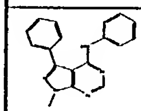
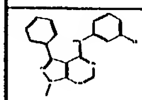
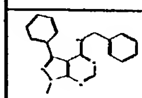
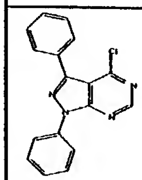
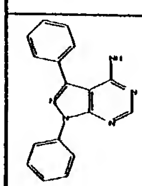
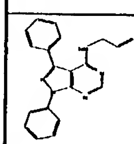
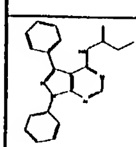
gg							
hh							
ii							
jj							
kk							
ll							
mm							
nn		>1000	0.510	0.4		<<6.5	
oo		>300	>10	>300			
pp		>300	>10	>300			

FIG. 12E

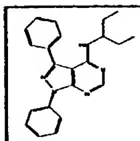
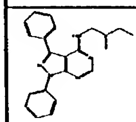
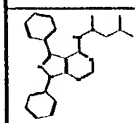
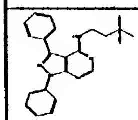
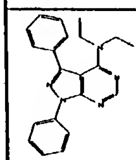
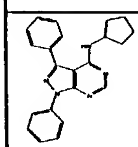
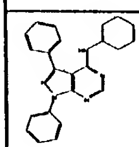
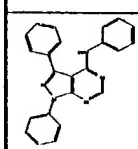
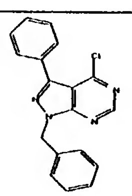
qq		>300	>10	>300			
rr		>300	>10	>300			
ss		>300	>10	>300			
tt		>300	>10	>300			
uu		>300	>10	>300			
vv		>300	>10	>300			
ww		>300	>10	>300			
xx		>300	>10	>300			
yy							

FIG. 12F

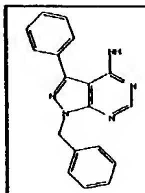
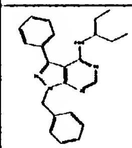
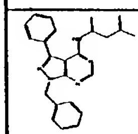
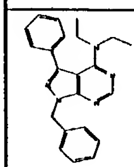
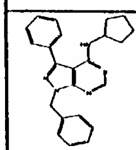
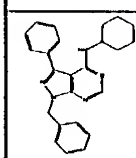
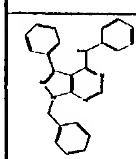
zz		<10	2.5	<<10			
aaa		>300	>10	>300			
bbb		>300	>10	>300			
ccc		>300	>10	>300			
ddd		>300	>10	>300			
eee		>300	>10	>300			
fff		>300	>10	>300			

FIG. 13A

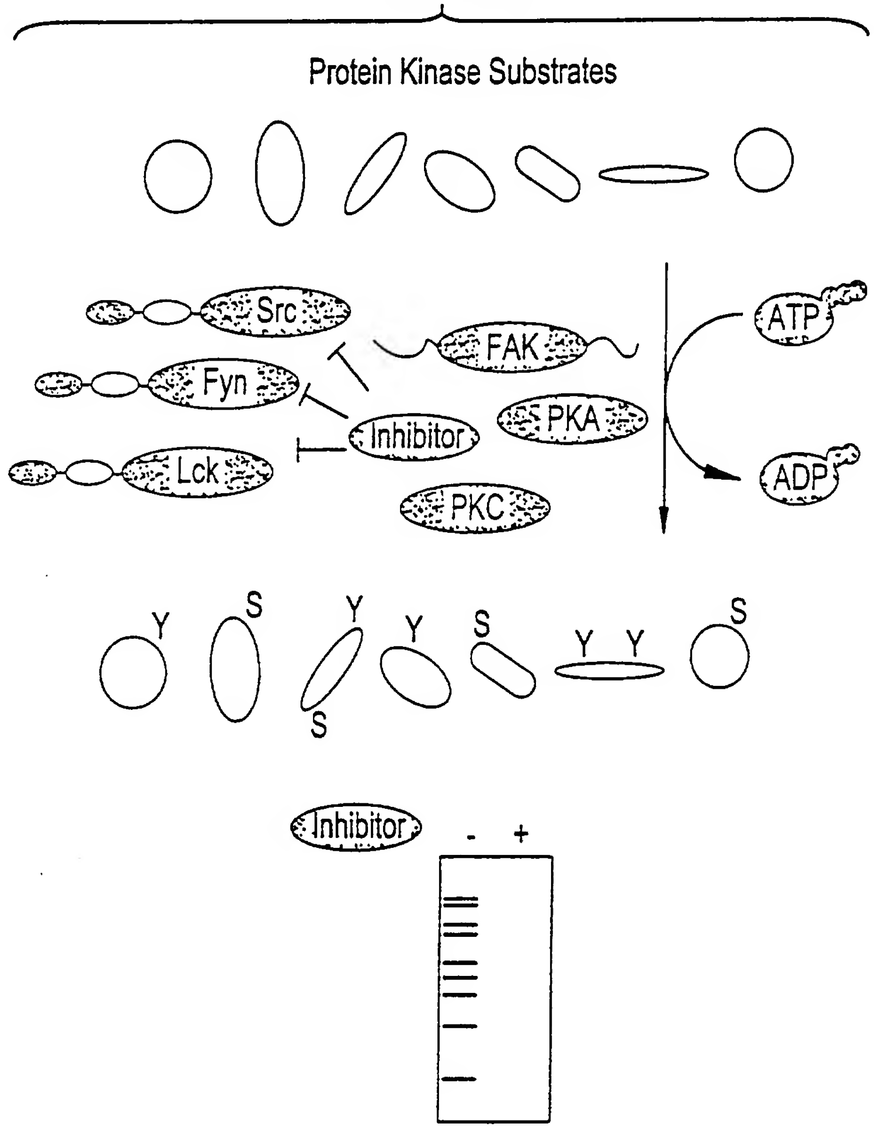


FIG. 13B

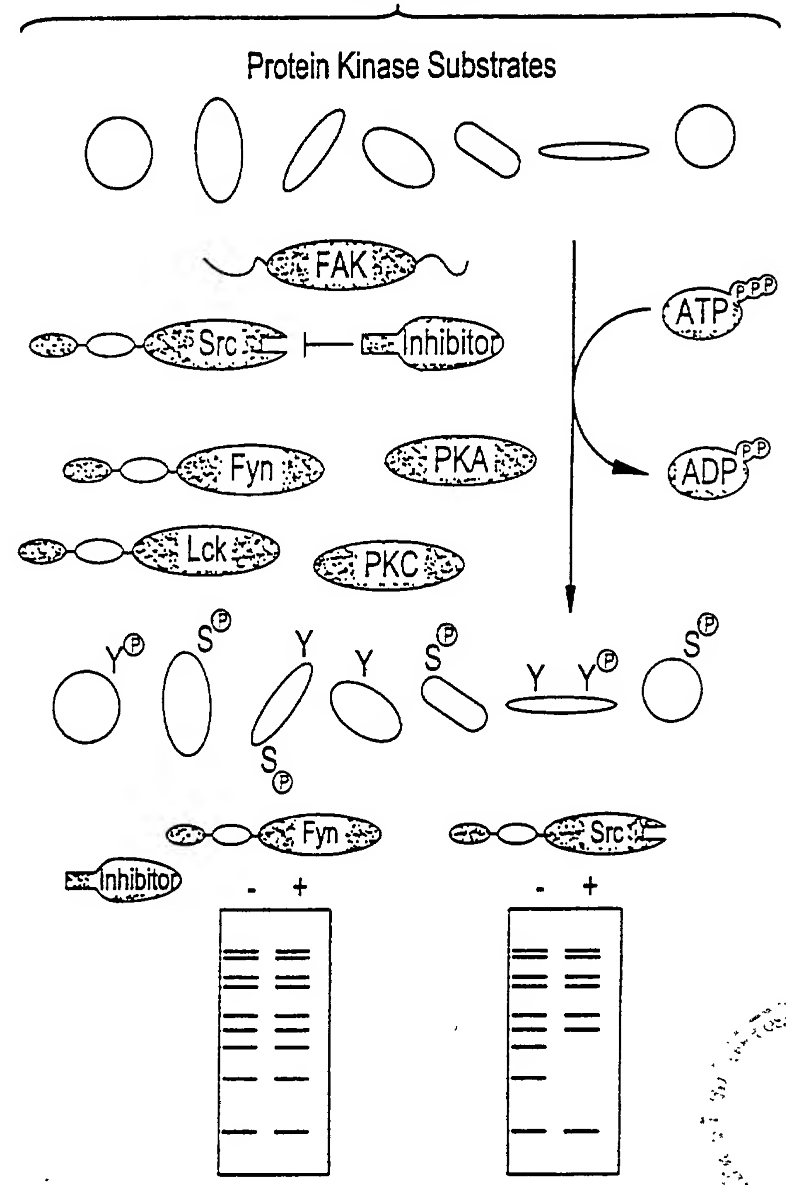
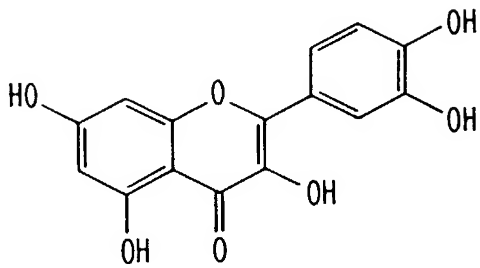
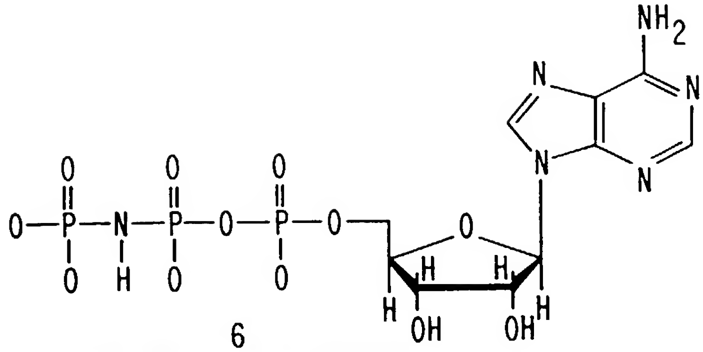


FIG. 15A



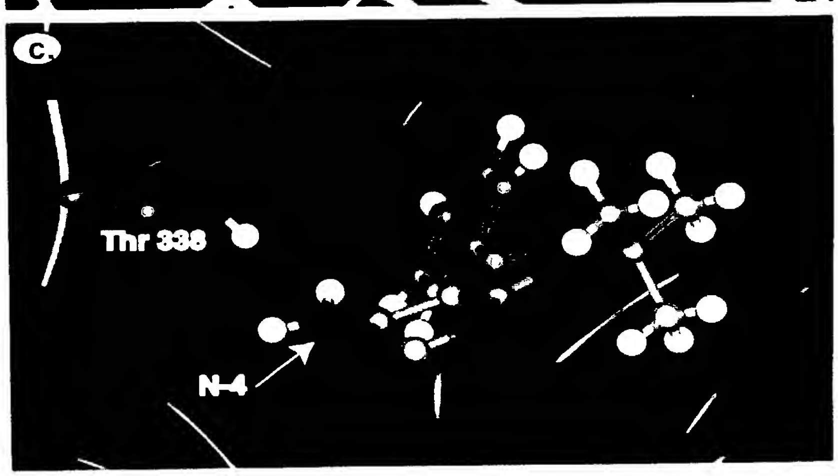
5



6

FIG. 15B

FIG. 15C



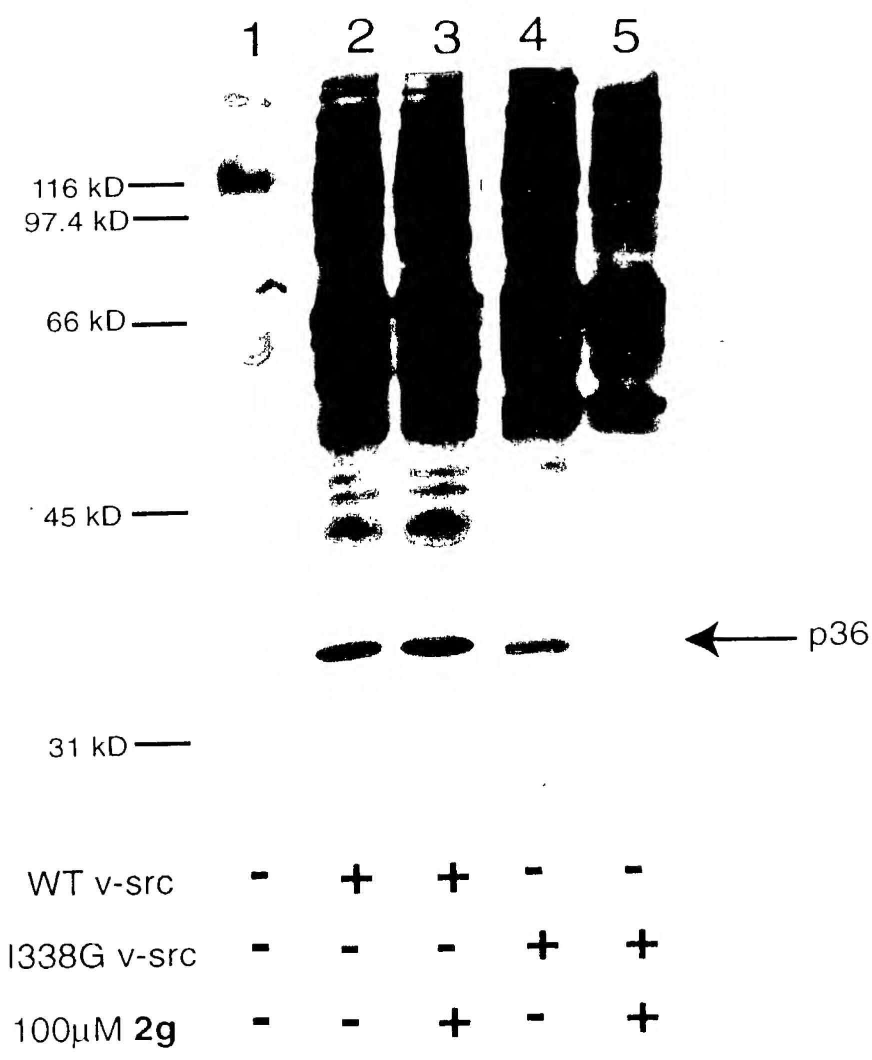

1
2
3
4
5
116 kD
97.4 kD
66 kD
45 kD
31 kD
p36
WT v-src - + + - -
I338G v-src - - - + +
100μM 2g - - + - +

FIG. 16

FIG. 17A

FIG. 17B

FIG. 17C

FIG. 17D

FIG. 17E

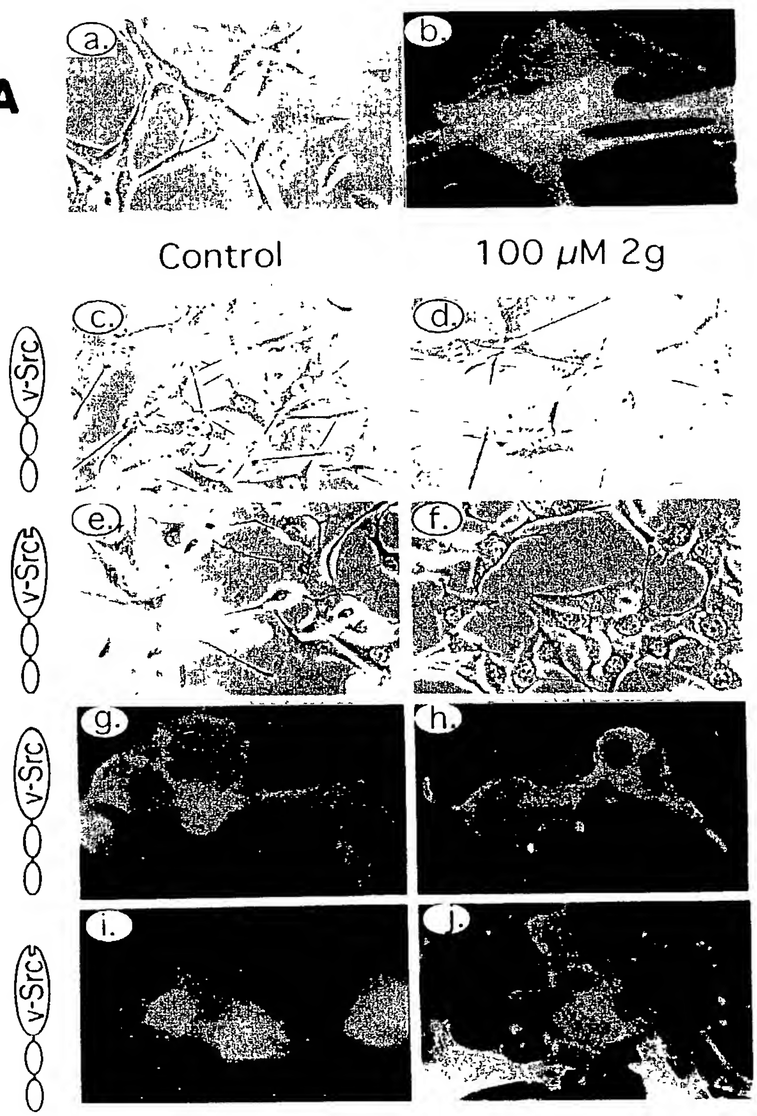
FIG. 17F

FIG. 17G

FIG. 17H

FIG. 17I

FIG. 17J



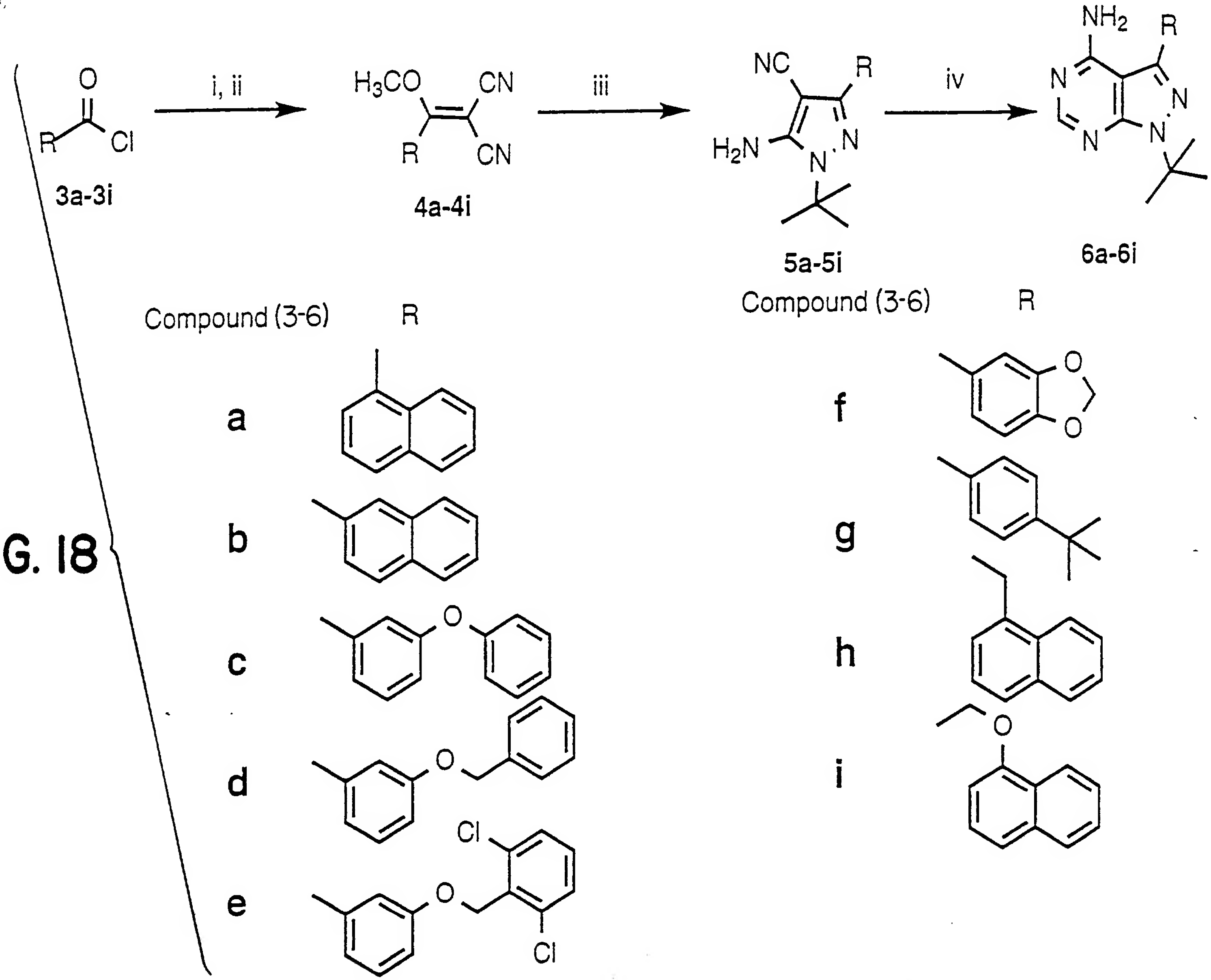

i, ii
iii
iv
3a-3i
4a-4i
5a-5i
6a-6i
Compound (3-6)
R
a
b
c
d
e
Compound (3-6)
R
f
g
h
i

FIG. 18

FIG. 20A

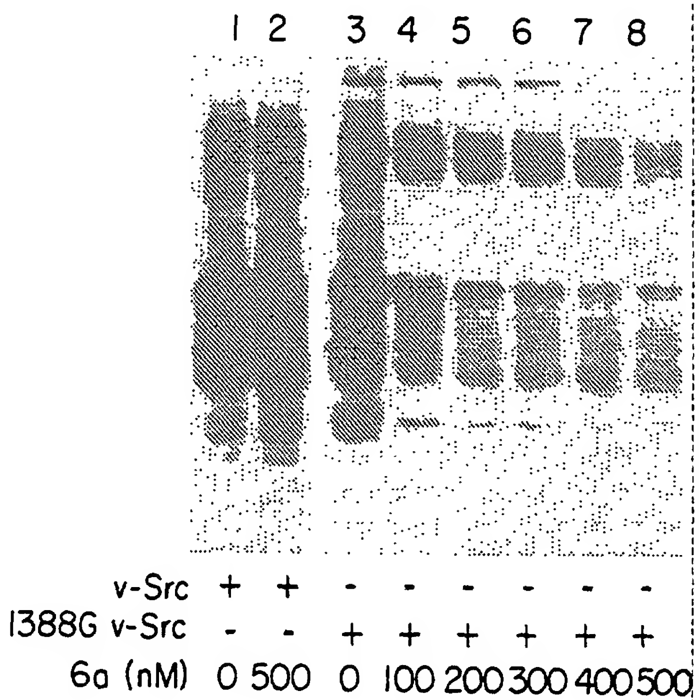


FIG. 20B

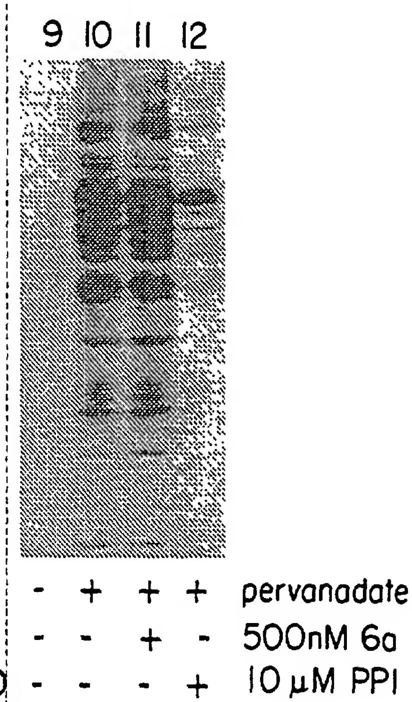


FIG. 21A

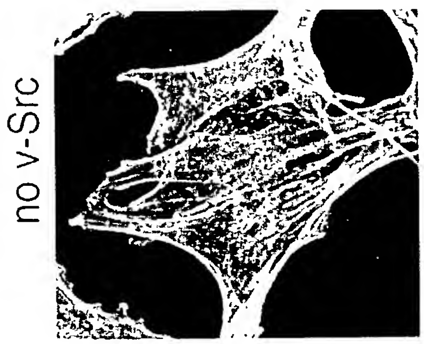


FIG. 21B

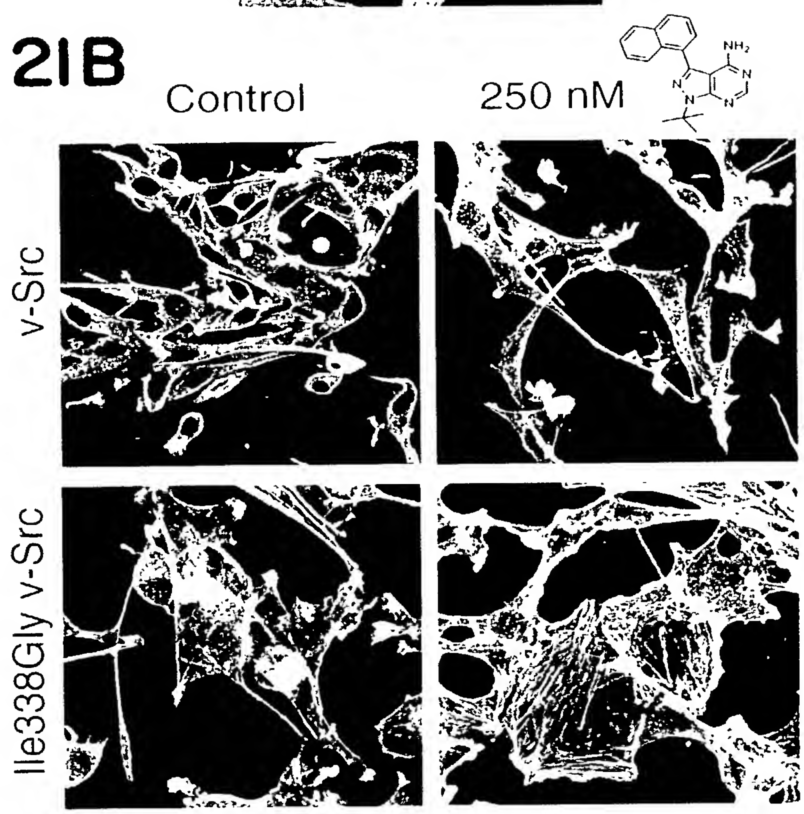
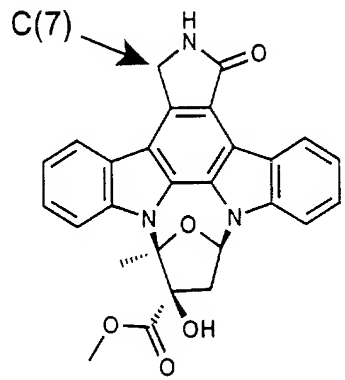
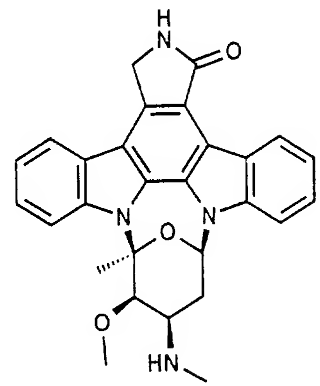


FIG. 22A



(+)-K252a (**1**)



(+)-Staurosporine (**2**)

FIG. 22B

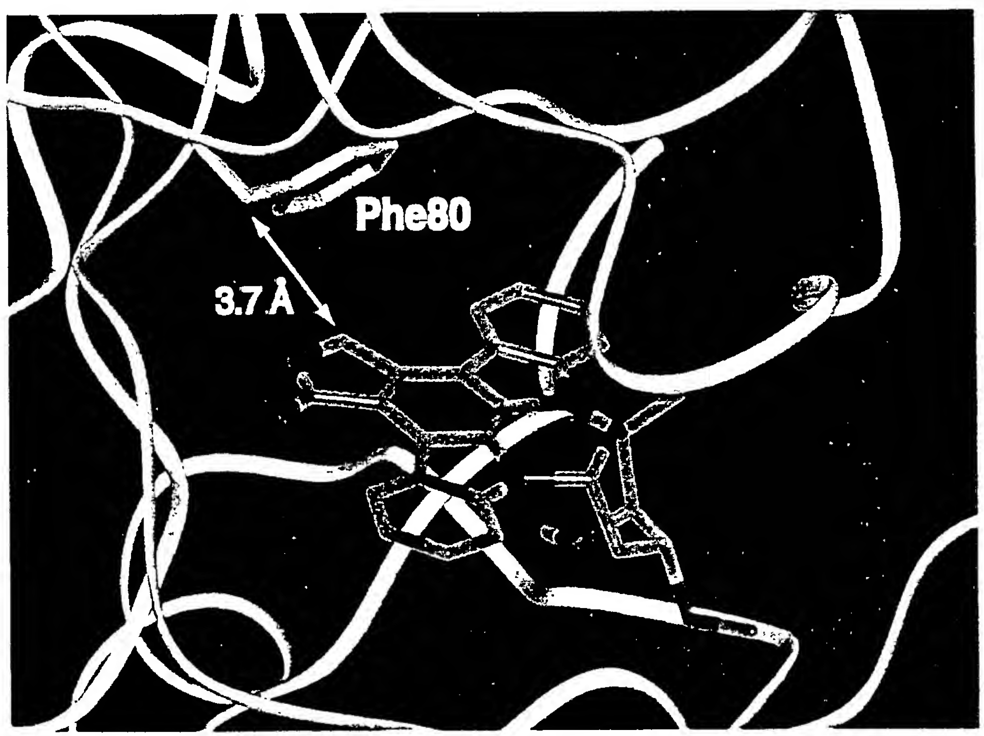


FIG. 23A

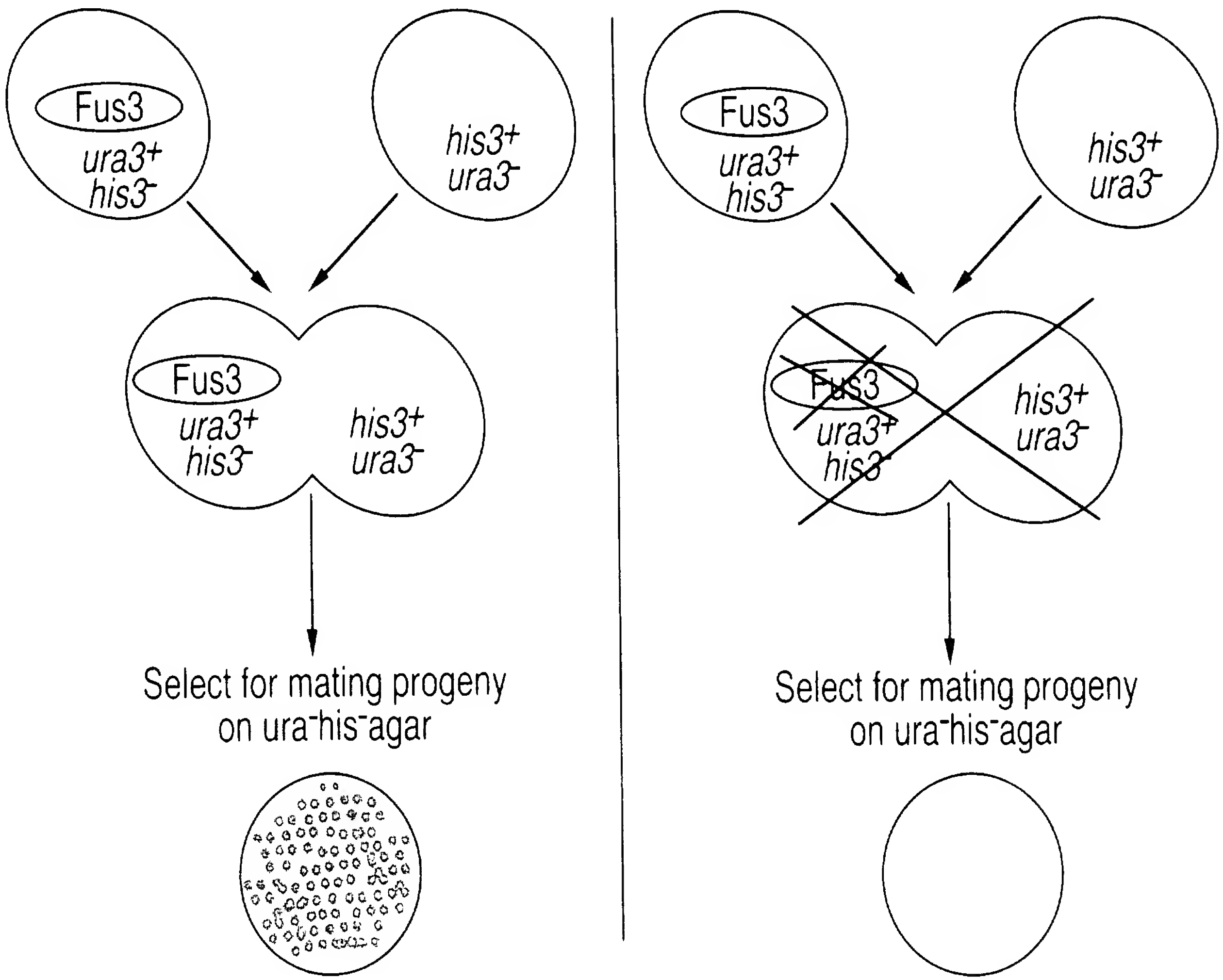

Fus3
ura3+
his3-
his3+
ura3-
Fus3
ura3+
his3-
his3+
ura3-
Select for mating progeny
on ura-his-agar
Fus3
ura3+
his3-
his3+
ura3-
Fus3
ura3+
his3-
his3+
ura3-
Select for mating progeny
on ura-his-agar

FIG. 23B

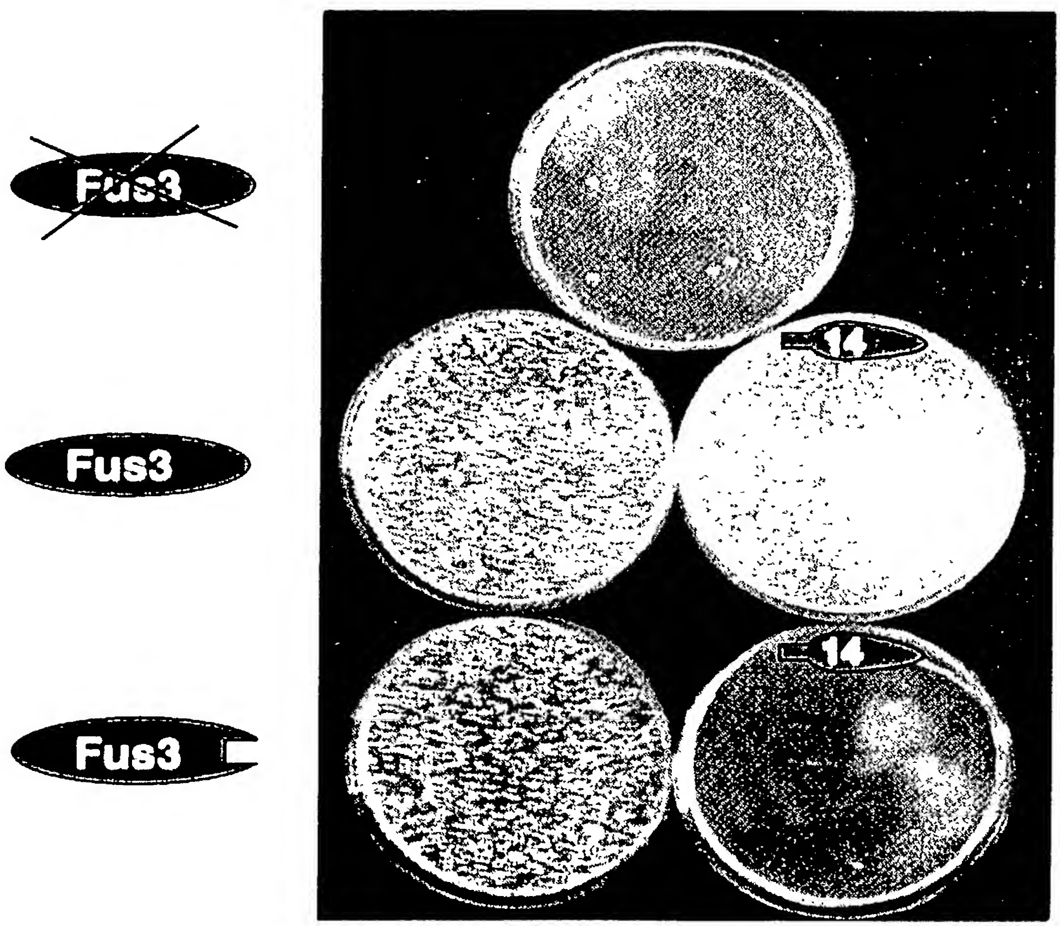
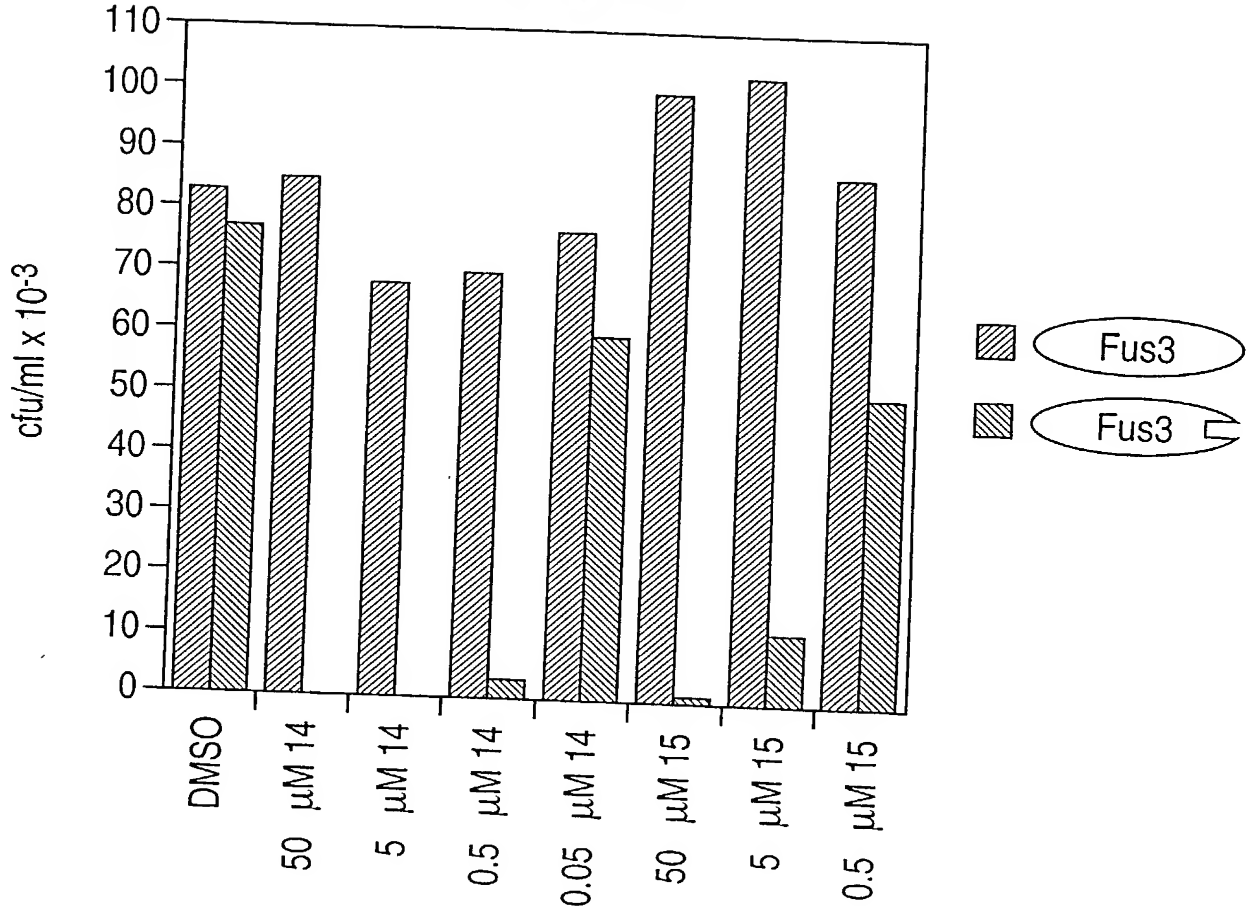
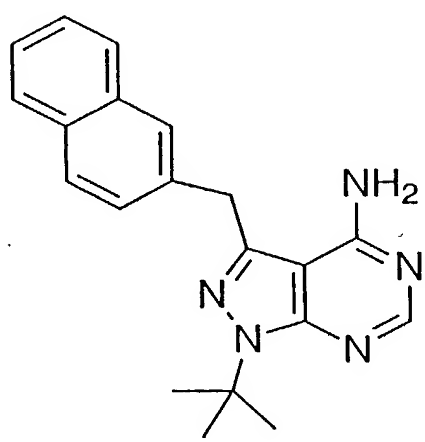


FIG. 23C



110
100
90
80
70
60
50
40
30
20
10
0
cfu/ml x 10-3
DMSO
50 µM 14
5 µM 14
0.5 µM 14
0.05 µM 14
50 µM 15
5 µM 15
0.5 µM 15
Fus3
Fus3

FIG. 24



6j

FIG. 25A

All *S. cerevisiae* genes (6,200)

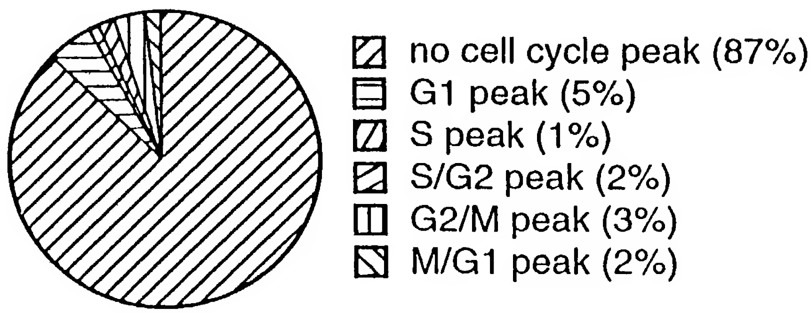
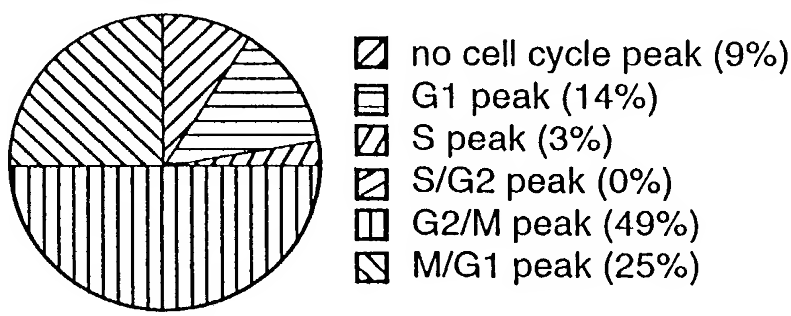


FIG. 25B

Decreases - 120 min (66)



Unreg

AMI1	-3
BAR1	-5.1
PUT4	-3.2
SUN4	-4.1
YBR077C	-2.6
YER067W	-5.5

G1

CTS1	-28.4
GPH1	-2.9
MFA1	-3.2
PRY3	-2.7
RME1	-3.1
RPC10	-41.2
SCW11	-16.4
YER124C	-9.8
YHR218W	-3

S

HTB2	-4.6
MET14	-2.9

G2/M

ALK1	-3.5
ATF2	-5.1
BNS1	-3.7
CDC20	-4.1
CDC5	-3
CLB2	-4.1
DBF2	-2.6
FAR1	-20.4
HST3	-4.1
MFA2	-6.9
MYO1	-3
PHO11	-4.9
PHO12	-5.9
PHO3	-21.5
PHO5	-10.6
PRY1	-3.2
RPI1	-2.7
SDL1	-3.8
SKN1	-2.5
STE2	-2.5
STE6	-5.8
SUR7	-2.5
SWI5	-3.1
UTH1	-2.5
WSC4	-6.9
YDR033W	-13.6
YIL158W	-3.1
YJL051W	-4
YLR254C	-4.2
YML119W	-4.1
YNL058C	-3.1
YRO2	-7.8

M/G1

AGA2	-6.5
EGT2	-28.3
FAA3	-4.7
GYP6	-2.5
IAH2	-3.4
ICS4	-4.7
MCM3	-2.6
PCL9	-4.9
PIR1	-3.7
PTS1	-3.5
SPI1	-2.6
YGP1	-5.5
YNL046W	-5.7
YNR067C	-19.4
YOR066W	-3.7
YOR264W	-4.7
YPL158C	-4.6

FIG. 25D

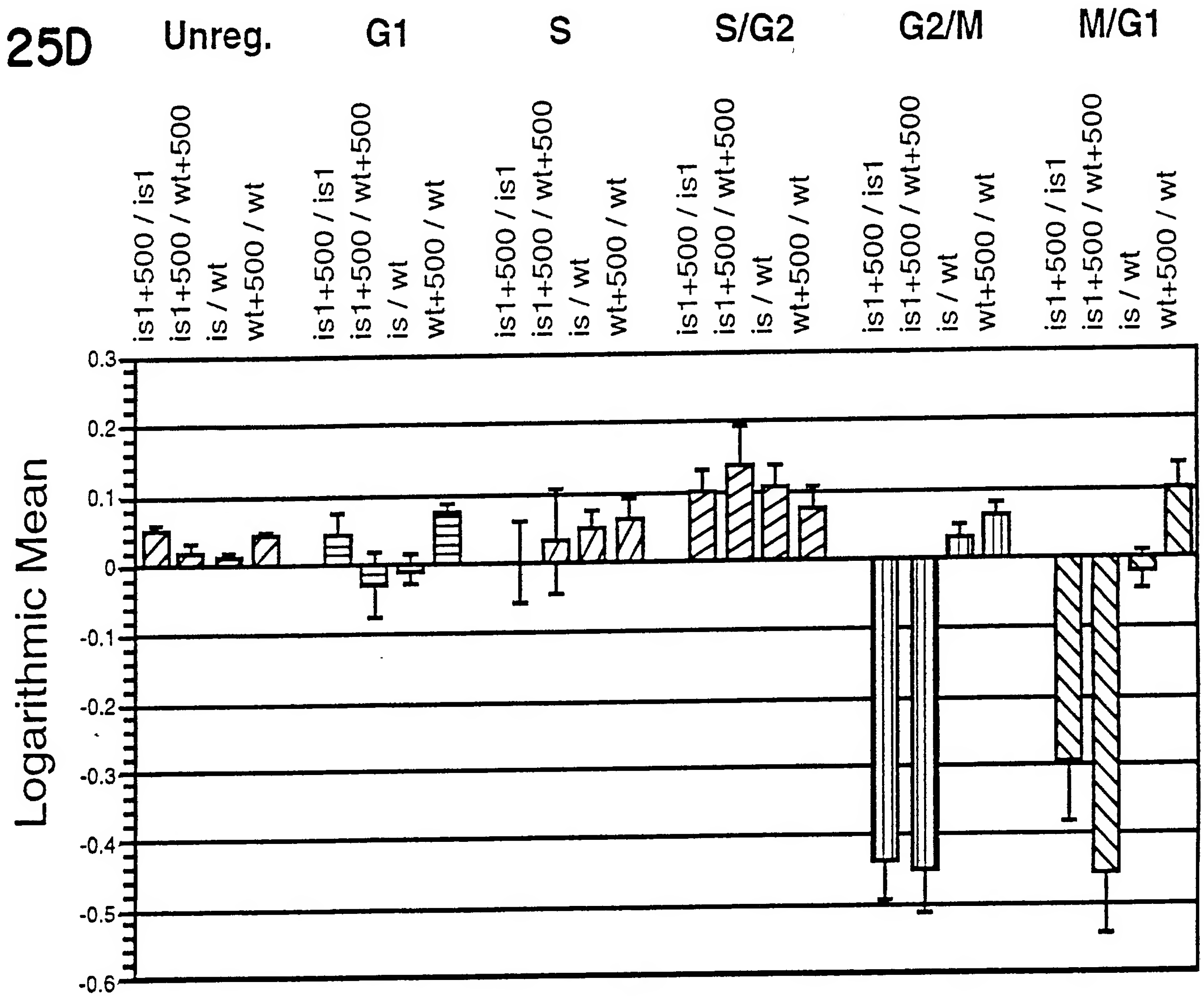

Unreg.
G1
S
S/G2
G2/M
M/G1
is1+500 / is1
is1+500 / wt+500
is / wt
wt+500 / wt
Logarithmic Mean
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6

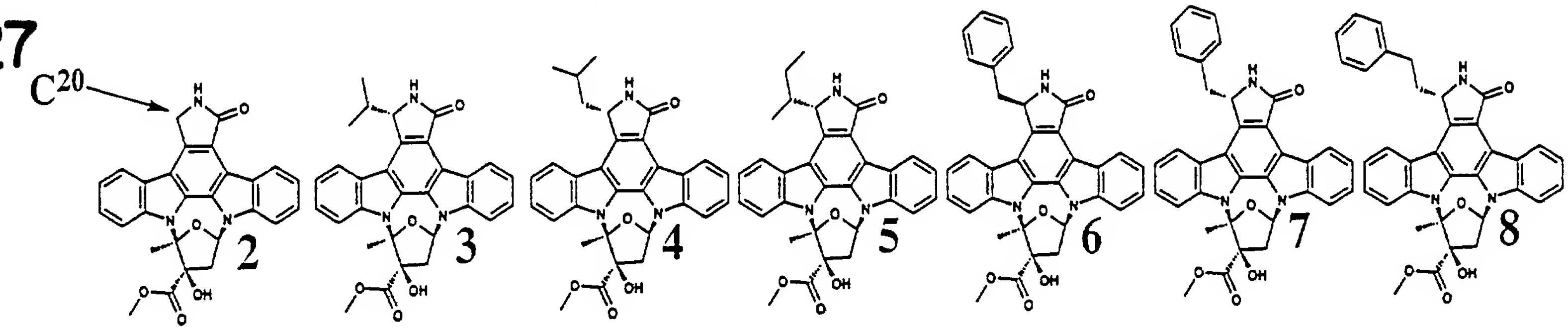
FIG. 26A

Protein Kinase	Kinase Family	Specificity	Cellular Function
v-Src	Src	Tyr	oncogenic transformation
c-Fyn	Src	Tyr	lymphocyte activation
c-Abl	Abl	Tyr	F-actin binding, transcription
CAMK II	calcium/calmodulin dependent	Ser/Thr	long-term potentiation, memory
CDK2	cyclin dependent	Ser/Thr	mammalian cell cycle progression
CDC28	cyclin dependent	Ser/Thr	*S. cerevisiae* cell cycle progression
Fus3	mitogen-activated	Ser/Thr	*S. cerevisiae* mating

FIG. 26B

```
                                                     338
                                                      ▼
v-Src     (318)RHEKLVQLYAMVSE----------EPIYIVIEYMSK--GSLLDFLKGEMGKY
Fyn       (319)KHDKLVQLYAVVSE----------EPIYIVTEYMNK--GSLLDFLKDGEGRA
Abl       (294)KHPNLVQLLGVCTRE---------PPFYIITEFMTY--GNLLDYLRECNRQE
CamK. II  (68) KHPNIVRLHDSISEE---------GHHYLIFDLVTG--GELFEDIVAREY
Cdk2      (59) NHPNIVKLLDVIHTE---------NKLYLVFEFLHQ---DLKKFMDASALTG
Cdc28     (66) KDDNIVRLYDIVHSDA--------HKLYLVFEFLDL---DLKRYMEGIPKDQP
Fus3      (67) KHENIITIFNIQRPDSFENF----NEVYIIQELMQT---DLHRVISTQM
```

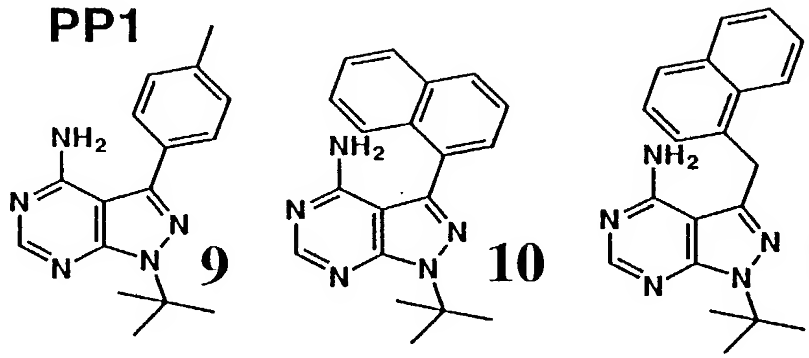
FIG. 27



	2	3	4	5	6	7	8
Wild Type							
v-Src	0.020	>33	>33	26	11	3.6	12
c-Fyn	0.17	>33	>33	>33	>33	30	>33
c-Abl	2.2	>33	>33	>33	>33	>33	>33
CDK2	0.020	29	22	5.8	10	1.0	3.7
CAMK II	0.054	2.7	1.7	0.82	0.49	0.13	0.54
Engineered							
v-Src		0.044	0.00023	0.092	0.019	0.0012	0.0021
c-Fyn		0.12	0.00055	0.30	0.057	0.0018	0.0043
c-Abl		>11	3.1	>11	>11	>11	9.6
CDK2		1.9	0.070	>3.7	10	0.15	0.037
CAMK II		2.3	0.95	>3.7	>1.2	0.39	0.0040

FIG. 28

PP1



9 10 11

	9	10	11
Wild Type			
v-Src	2.2	1.0	28
c-Fyn	0.050	0.60	1.0
c-Abl	0.30	0.60	3.4
CDK2	22	18	29
CAMK II	17	22	24
Engineered			
v-Src		0.0015	0.0043
c-Fyn		0.0065	0.0032
c-Abl		0.0070	0.12
CDK2		0.015	0.0050
CAMK II		0.097	0.0080